

### **Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

### **Listing of Claims:**

1. (currently amended) A method for determining the presence of coliform bacteria in a drinking water sample comprising the steps of:
  - a) separating said bacteria from said drinking water sample using a first filter means;
  - b) ~~culturing said bacteria in~~ transferring said bacteria from said first filter means to a broth comprising nutrients for supporting growth of said bacteria and an inducing agent for inducing enzyme production in said bacteria and culturing said bacteria in said broth;
  - c) separating said bacteria from said broth using a second filter means;
  - d) exposing said bacteria to a lysing agent;
  - e) incubating a chemiluminogenic substrate of said enzyme with said enzyme to cause cleavage of said substrate, thereby producing a luminescent product;
  - f) initiating light emission by exposing said luminescent product to an enhancing agent that excludes water from a cleavage site of said substrate to prevent quenching of said light emission by water molecule induced protonation; and,
  - g) detecting said light emission to thereby determine the presence of said bacteria in said sample.
2. (cancelled).
3. (previously presented) The method of claim 1, wherein said bacteria are separated from said broth before being exposed to said lysing agent.
4. (previously presented) The method of claim 1, wherein said bacteria are on said second filter means during exposure to said lysing agent.
5. (previously presented) The method of claim 1, wherein said light emission is detected by means of a luminometer.
6. (previously presented) The method of claim 1, wherein said luminescent product is on said second filter means during detection of said light emission.
7. (previously presented) The method of claim 5, wherein said luminescent product is on said second filter means during detection of said light emission and wherein said second filter means is within said luminometer during detection of said light emission.

8. (cancelled).
9. (previously presented) The method of claim 1, wherein said culturing is at a temperature of about 22 to 45 °C for about 2 to 10 hours.
10. (previously presented) The method of claim 1, wherein said chemiluminogenic substrate comprises 1,2-dioxetane.
11. (previously presented) The method of claim 1, wherein said enhancing agent comprises quaternary ammonium homopolymer.
12. (cancelled).
13. (previously presented) The method of claim 1, wherein said enzyme is  $\beta$ -D-galactosidase.
14. (original) The method of claim 13, wherein said culturing is at a temperature of about 35 °C for about 5 hours.
15. (previously presented) The method of claim 13, wherein said inducing agent comprises isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG), lactose, or a combination thereof.
16. (previously presented) The method of claim 13, wherein said substrate comprises 3-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo-[3.3.3.3<sup>3,7</sup>]decan}-4-yl)phenyl  $\beta$ -D-galactopyranoside.
17. (cancelled).
18. (cancelled).
19. (previously presented) The method of claim 1, wherein said enzyme is  $\beta$ -D-glucuronidase.
20. (original) The method of claim 19, wherein said culturing is at a temperature of about 44.5 °C for about 9 hours.
21. (previously presented) The method of claim 19, wherein said inducing agent comprises methyl- $\beta$ -D-glucuronide (Met-Glu).
22. (previously presented) The method of claim 19, wherein said substrate comprises sodium 3-(4-methoxyspiro{1,2-dioxetane-3-,2'-(5'-chloro)-tricyclo-[3.3.1.1<sup>3,7</sup>]decan}-4-yl)phenyl  $\beta$ -D-glucuronate.
23. (cancelled).
24. (cancelled).

25. (previously presented) The method of claim 1, wherein said lysing agent comprises toluene, successive freeze thaw cycles, a change of pressure, lysozyme, a detergent, octylphenoxypolyethoxyethanol nonionic surfactant, potassium dihydrogen phosphate, polymyxin-B, or a combination thereof.
26. (cancelled).
27. (previously presented) The method of claim 1, wherein said broth further comprises an inhibiting agent for inhibiting the growth of non-target organisms
28. (cancelled).
29. (currently amended) A method for determining the quantity of coliform bacteria in a drinking water sample comprising the steps of:
- a) separating said bacteria from said drinking water sample using a first filter means;
  - b) ~~culturing said bacteria in~~ transferring said bacteria from said first filter means to a broth comprising nutrients for supporting growth of said bacteria and an inducing agent for inducing enzyme production in said bacteria and culturing said bacteria in said broth;
  - c) separating said bacteria from said broth using a second filter means;
  - d) exposing said bacteria to a lysing agent;
  - e) incubating a chemiluminogenic substrate of said enzyme with said enzyme to cause cleavage of said substrate, thereby producing a luminescent product;
  - f) initiating light emission by exposing said luminescent product to an enhancing agent that excludes water from a cleavage site of said substrate to prevent quenching of said light emission by water molecule induced protonation; and,
  - g) measuring said light emission to obtain a light measurement corresponding to ~~the a~~ a quantity of said enzyme and comparing said measurement to a previously prepared calibration curve correlating a series of light signals with a known quantity of enzyme or bacteria producing each signal in the series to thereby determine the quantity of said bacteria in said sample.
30. – 85. (cancelled).
86. (currently amended) A kit for determining the presence of coliform bacteria in a drinking water sample comprising ~~the steps of:~~
- a) a first filter means for separating said bacteria from said drinking water sample using a first filter means;
  - b) a broth comprising nutrients for supporting growth of said bacteria for use in culturing said bacteria at a temperature of about 22 to 45 °C for about 2 to 10 hours in a broth comprising nutrients for supporting growth of said bacteria and after transferring said bacteria from said first filter means to

- said broth, said broth including an inducing agent comprising isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) or methyl- $\beta$ -D-glucuronide (Met-Glu) for inducing production of an enzyme in said bacteria;
- c) a second filter means for separating said bacteria from said broth using a second filter means; followed by,
  - d) a lysing agent for exposure to exposing said bacteria on said second filter means following separation to a lysing agent comprising polymyxin-B;
  - e) incubating a chemiluminogenic substrate of said enzyme comprising 1,2-dioxetane for incubation with said enzyme to cause cleavage of said substrate, thereby producing a luminescent product on said second filter means;
  - f) initiating light emission by exposing said luminescent product to an enhancing agent comprising quaternary ammonium homopolymer that excludes water from a cleavage site of said substrate to prevent quenching of said light emission by water molecule induced protonation, said enhancing agent for initiating light emission upon exposure to said luminescent product; and,
  - g) a luminometer for detecting or measuring said light emission using a luminometer by placing said second filter means with said luminescent product within said luminometer to thereby determine the presence or quantity of said bacteria in said sample; and,
  - h) instructions for use of items a) to g) in accordance with their previously described functions.